MODELLING OF ENFETS FOR THE CREATININE DETECTION

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Abstract

Creatinine enzymatic field effect transistors (C-EnFETs) realised from a pH-sensitive field effect transistor (pH-ISFET) have been modelled by taking into account the enzymatic reaction, the diffusion phenomena of the main chemical species in the electrolyte, the related acid/basic chemical reactions in watery solution and the detection properties of the pH-ISFET sensitive gate. Thus, the concentrations of the main chemical species have been characterized near the sensitive gate, and the C-EnFET micro sensor detection properties have been studied according to the most influential parameters. A good fit has been shown between modelling and experimental results. The model has been developed for the optimization of creatinine-EnFETs in the field of haemodialysis, but is also fully compatible for other EnFETs based on pH-ISFET-metry.

Keywords: Modelling, EnFETs, creatinine detection

1. Introduction

Since its development in the sixties for patients suffering from chronic end-stage kidney failure, haemodialysis has been in constant evolution in order to improve health's care and life expectancy. Today, in order to proceed on its way, the dialysis efficiency must be known precisely by monitoring urea and creatinine concentration during haemodialysis treatment.

This goal will be achieved by developing integrated, disposable, low cost and reliable biochemical sensors fully adapted to medical analysis. These requirements can be reached using chemical field effect transistor (ChemFET) micro sensors [1]. Indeed, enzymatic layers have been thoroughly studied for the development of pH-ISFET-metry, i.e. for the adaptation of pH-sensitive ion sensitive field effect transistor (pH-ISFET) to biochemical detection. By using enzymes responsible for acid or basic chemical production, enzymatic field effect transistor (EnFET) based micro sensors have been realized for many applications including urea and creatinine detection [2-7]. However, in order to optimize the EnFETs micro sensors for an industrial haemodialysis application in terms of cost, their detection phenomena and properties must be modelled and understood.

Thus, this paper deals with the modelling of a creatinine-sensitive EnFET (C-EnFET) micro sensor using poly vinyl alcohol (PVA) based enzymatic layers.

2. Modelling

Creatinine EnFET detection principle is based on the use of a SiO_2/Si_3N_4 pH-ISFET adapted to enzymatic detection thanks to a PVA creatinine deiminase rich, enzymatic layer [7]. The Si_3N_4/PVA/electrolyte (water) sensitive structure has been described according to figure 1. The C-EnFET detection properties that were modelled are the enzymatic reaction, the diffusion phenomena of the main chemical species, i.e. creatinine $C_4H_7N_3O$ and ammonia NH_3, in the electrolyte, the NH_4^+/NH_3 related acid/basic reactions into aqueous solution, and finally the pH-detection properties of the silicon nitride Si_3N_4 ISFET gate.

A. Modelling of the creatinine deiminase enzymatic reaction

The creatinine deiminase (CD-ase) enzymatic reaction is responsible for the creatinine hydrolysis into aqueous solution (equation 1):

$$\text{creatinine} \quad + \quad H_2O \quad \text{------>} \quad \text{N-methyl hydantoïn} \quad + \quad NH_3 \qquad (1)$$

This enzymatic reaction has been modelled using the Michaelis – Menten equation:

$$a = a_M \frac{[S]}{[S] + K_M} \qquad (2)$$

where a is the enzyme activity, a_M is the maximal activity ($a_M = 16.67 \times 10^{-9}$ mol/s for 1 CD-ase unit), [S] is the creatinine concentration in solution and K_M is the creatinine deiminase Michaelis constant.

In the following, since creatinine and N-methyl hydantoïn are not known to have any acid/basic properties, their consumption/production have been assumed to have no influence on the C-EnFET detection properties. Furthermore, no enzyme activity dependence with pH has been taken into account.

B. Modelling of the diffusion phenomena of creatinine and ammonia in the electrolyte

Diffusion phenomena of the most influential chemical species, i.e. creatinine $C_4H_7N_3O$ (named S for "substrate") and ammonia NH_3, into water have been modelled using the Fick law:

$$\begin{cases} \dfrac{\partial [S](x,t)}{\partial t} = D_S \dfrac{\partial^2 [S](x,t)}{\partial x^2} - g(x,t) \\ \dfrac{\partial [NH_3]_p(x,t)}{\partial t} = D_{NH_3} \dfrac{\partial^2 [NH_3]_p(x,t)}{\partial x^2} + g(x,t) \end{cases} \qquad (3)$$

where $[S](x,t)$ is the creatinine concentration, $[NH_3]_p(x,t)$ is the ammonia global concentration produced, D_s and D_{NH3} are the diffusion coefficients of creatinine and ammonia molecules into water respectively.

Since the PVA enzymatic layer is composed of at least 93% of water [8], the D_s and D_{NH3} values have been assumed constant for all layer thicknesses (parameter x in figure 1). Their values ($D_s = 1.35\ 10^{-5}$ cm^2/s and $D_{NH3} = 2.54\ 10^{-5}$ cm^2/s) have been estimated according to the Einstein equation:

$$D = A \frac{kT}{\sqrt[3]{M}} \qquad (4)$$

where A is a parameter related to the electrolyte, k is the Boltzman constant, T is the absolute temperature and M is the molar mass of the studied molecule.

The $g(x,t)$ parameter represents the enzymatic consumption/production phenomena per time unit. Since the creatinine deiminase enzymatic reaction occurs only in the PVA layer, $g(x,t)$ has been chosen equal to zero in the electrolyte (figure 1). Thus, according to equation (1), $g(x,t)$ is given by:

$$\begin{cases} 0 \le x \le e_{PVA} : g(x,t) = a_M n_{enz} \dfrac{[S](x,t)}{[S](x,t) + K_M} \\ e_{PVA} \le x : g(x,t) = 0 \end{cases} \quad (5)$$

where e_{PVA} is the PVA thickness and n_{enz} is the number of enzymatic units per volume unit in the PVA layer.

Thus, by resolving the system formed by equations (3) and (5), the [S](x,t) and $[NH_3]_p(x,t)$ concentrations can be determined.

C. Modelling of the acid/basic chemical reaction in the electrolyte

The production of ammonia NH_3 in aqueous solution is known to be responsible for a pH variation according to the acid/basic relation:

$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^- \quad (6)$$

Thus, the pH value, i.e. the $[H^+]$ ion concentration, can be calculated by studying the standard chemical reactions related to the NH_4^+/NH_3 acid/base couple (equations 7):

$$\begin{cases} [NH_4^+] + [NH_3] = [NH_3]_p \\ K_a = \dfrac{[NH_3][H_3O^+]}{[NH_4^+]} \\ K_e = [OH^-][H_3O^+] \\ [NH_4^+] + [H_3O^+] = [OH^-] + C_t \end{cases} \quad (7)$$

where K_a and K_e are respectively the NH_4^+/NH_3 acidity constant and the water ionic product ($K_a = 5.62\ 10^{-10}$ mol/l and $K_e = 10^{-14}$ $(mol/l)^2$).

C_t is a constant term describing the acid/basic properties of the initial solution. For a standard initial solution with a given pH_0 value, C_t is given by:

$$C_t = 10^{pH_0} - 10^{(pK_e - pH_0)} \tag{8}$$

Assuming that time constants of the different acid/basic reactions (equations 7) are very low compared to those of the diffusion phenomena (equations 3), the $[H_3O^+](x,t)$ concentration, i.e. the pH(x,t) function, can be related to the ammonia global concentration produces by the enzymatic reaction $[NH_3]_p(x,t)$ according to equations 9:

$$\begin{cases} \dfrac{[H_3O^+](x,t)[NH_3]_p(x,t)}{[H_3O^+](x,t)+K_a} + [H_3O^+](x,t) - \dfrac{K_e}{[H_3O^+](x,t)} - C_t = 0 \\ pH(x,t) = -\log[H_3O^+](x,t) \end{cases} \tag{9}$$

D. Modelling of the pH-ISFET response

Finally, the C-EnFET threshold voltage V_T is related to the pH at the silicon nitride Si_3N_4 surface according to the simplified site-binding model [9,10]:

$$V_T = V_{T0} + s_0[pH(0,+\infty) - pH_{pzc}] \tag{10}$$

where V_{T0} is the threshold voltage of the corresponding field effect transistor, s_0 is the pH-ISFET sensitivity (given theoretically by the Nernst law or estimated experimentally), $pH(0,+\infty)$ is the pH at the silicon nitride gate surface when the diffusion phenomena "steady state" is reached, and pH_{pzc} is the point of zero charge (pH_{pzc} has been estimated around 4 for Si_3N_4 [10,11]).

In the following, since the V_{T0} value is only related to the pH-ISFET technological fabrication [12], it is of no influence concerning the C-EnFET detection properties and it will not be taken into account, i.e. it will be chosen equal to zero.

3. Results and discussion

A. Study of the main chemical species concentrations

Figures 2 and 3 represent typical variations of the creatinine and ammonia concentrations [S](x,t) and $[NH_3](x,t)$ near the sensor surface. They clearly show the creatinine consumption as well as the ammonia production. However, the main influence of the enzymatic reaction is given by the pH variations pH(x,t) in proximity to the pH-ISFET sensitive surface (figure 4). It appears that the pH local increase reaches millimetric distances from the sensor surface and that a "steady state" is evidenced at the sensor pH-sensitive surface after around 10 seconds (figure 5). Thus, due to the maximal limit $pH(0,+\infty)$, the C-EnFET detection properties can be thoroughly studied (equation 10).

B. Study of the most influential parameters

Firstly, the influence of the PVA thickness e_{PVA} has been studied. For e_{PVA} values ranging between 1 and 10 microns [8], no variation of the sensor detection properties has been found. Indeed, in all cases, the PVA thickness is insignificant compared to the typical lengths of the pH increase (figure 4). Therefore, the enzymatic reaction can be considered as a surface phenomenon occurring at the sensor sensitive gate. In the following, the PVA thickness has been fixed according to profilometric experimental measurements [8].

According to theoretical equations, the most influential parameters are the number of creatinine deiminase enzymatic units per volume unit in the PVA layer n_{enz}, the creatinine deiminase Michaelis constant K_M, and the solution initial pH value pH_0. Their influences on the C-EnFET sensor detection properties have been studied on ranges appropriate with creatinine detection in the field of haemodialysis (main values are given in bold characters):

- [creatinine] (mol/L): [10^{-7} - 10^{-2}]
- K_M (mol/L): [10^{-4} - 10^{0}] (**3.5 10^{-3}**)
- n_{enz} (unit/cm^3): [10^{1} - 10^{6}] (**10^{4}**)
- pH_0: [6 - 8] (**7.5**)

Figure 6 represents typical C-EnFET responses with creatinine deiminase Michaelis constant K_M. For the lowest values, saturation of the creatinine detection properties is evidenced. These saturation phenomena should be related with the creatinine diffusion from the electrolyte towards the PVA enzymatic layer. They also allow to define the C-EnFET detection limit around 10^{-7} mol/L. Finally, figure 6 shows that the K_M increase is responsible for the shift of the C-EnFET detection range towards the higher creatinine concentration.

In the same way, figure 7 represents typical C-EnFET responses with creatinine deiminase enzymatic units per volume unit in the PVA layer n_{enz}. As n_{enz} is decreased towards the lower extreme, creatinine detection properties are lost. Conversely, as n_{enz} is increased towards the highest extreme, the saturation phenomena of creatinine detection are highlighted. These responses were predicted by equation (5).

Finally, since the studied enzymatic detection principle is based on pH-metry, it depends on the solution initial pH value pH_0 (equation 8). In fact, it appears that the pH_0 parameter influences the C-EnFET detection properties only for the lowest creatinine concentrations (figure 8). For the haemodialysis application, this is no drawback since the pH_0 value for human dialysate is known to be constant around 7.4.

Taking into account the whole enzymatic reaction, it appears that the K_M and n_{enz} parameters allow the definition of C-EnFET detection range and sensitivity. For instance, for a creatinine deiminase provided by Sigma (from micro-organism EC 3.5.4.21, $K_M \approx 3.5\ 10^{-3}$ mol/L) and tested for the realisation of creatinine EnFETs [7], figure 7 define clearly the optimal n_{enz} value in term of cost, while maintaining quasi-linear response and the highest sensitivity (≈ 45 mV/pCreatinine) for the concentration range appropriate to haemodialysis (10^{-5} - 10^{-3} mol/L). In this case, the optimal value is for $n_{enz} \approx 10^4$ unit/cm^3.

C. Comparison between modelling and experience

In order to validate the creatinine sensor model, it was compared to experimental results from related literature. In fact, in order to take into account the threshold voltage non reproducibility from one EnFET to another (for technological reasons), its variations δV has been studied as a function of the creatinine concentration, taking the lowest value ($\approx 2\ 10^{-5}$ mol/L) as reference (figure 9).

In previous works [7], different sensor responses had been obtained by using a creatinine deiminase provided by Sigma. According to our experimental procedures, the main detection parameters can be resumed as following:

- $K_M \approx 3.5\ 10^{-3}$ mol/L
- $n_{enz} \approx 2\ 10^3$ unit/cm^3
- $pH_0 \approx 7.5$
- $s_0 \approx 50$ mV/pH

Since K_M, pH_0 and s_0 values are directly related to the enzyme, the initial solution and the pH-ISFET characteristics respectively, experimental and modelling results have been fitted thanks to the n_{enz} parameter, in good agreement with the experimental value (figure 9):

- #1: $n_{enz} \approx 2\ 10^3$ unit/cm^3
- #2: $n_{enz} \approx 10^3$ unit/cm^3

Thus, according to the model, the experimental measurement discrepancy evidenced in [7] has to be related to the n_{enz} parameter and therefore to the creatinine deactivation with processing and/or storage conditions [8].

All in all, a good fit has been obtained with experimental results, validating the whole model of the Creatinine-EnFET micro sensor.

4. Conclusion

The creatinine EnFET detection principle based on pH-ISFET-metry has been modelled by taking into account the enzymatic reaction, the diffusion phenomena of the main chemical species in the electrolyte, the related acid/basic chemical reactions into aqueous solution, and finally the detection properties on the pH-ISFET sensitive gate. Thus, it has been possible to characterize the concentration variations of the main interfering chemical species near the sensor surface, and to define the most influential parameters on the C-EnFET micro sensor detection properties. The model has been compared with experimental results and a good agreement has been evidenced for the concentration range appropriate to haemodialysis (10^{-5} - 10^{-3} mol/L).

This modelling enables a real understanding of the EnFETs detection principle based on pH-ISFET-metry. It opens solution for improving the EnFETs sensor reliability, taking especially into account biochemical aspects related to the enzyme processing, storage or ageing. It has first been developed for the detection of creatinine in the field of haemodialysis, but is fully compatible with other similar enzymatic detection. It will be soon applied to the urease enzyme and the optimization of urea-EnFETs still for haemodialysis applications.

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Biographies

Pierre Temple-Boyer was born on October 25, 1966. He received his Master's Degree in electronic engineering from the Ecole Supérieure d'Electricité (Paris – France) in 1990. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 1992 and received the PhD degree from the Institut National des Sciences Appliquées de Toulouse (France) in 1995. Since then, as a senior researcher, he has been working on the development of micro- and nanotechnologies.

Julie Le Gal was born on April 16, 1980. She received her Master's Degree in microelectronics from the Université Paul Sabatier de Toulouse (France) in 2005. She joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 2005. She is working on the development of ChemFETs micro sensors for biochemical applications.

Marie-Laure Pourciel was born on July 25, 1976. She received her Master's Degree in physics from the Université Paul Sabatier de Toulouse (France) in 1999 and her Diplome d'Etudes Approfondies in Biotechnology from the Institut National des Sciences Appliquées de Toulouse (France) in 2000. She joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 2000 and received the PhD degree the Institut National des Sciences Appliquées de Toulouse (France) in 2004. Since then, she has been working on the development of pH-ISFET-metry deviated techniques for medical applications.

William Sant was born on May 18, 1969. He received his Master's Degree in electronics in 1996 and his Diplome d'Etudes Approfondies in Microelectronics from the Université Paul Sabatier de Toulouse (France) in 1999. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes of the French Centre National de la Recherche Scientifique (LAAS-CNRS) in 2000 and received the PhD degree from the Université Paul Sabatier de Toulouse (France) in 2004. Since then, he has been working on the development of ChemFETs micro sensors for medical applications.

Augustin Martinez was born the 24th of May 1942. He joined the Laboratoire d'Architecture et d'Analyse des Systèmes from the french Centre National de la Recherche Scientifique (LAAS-CNRS) in 1966 and received his Doctorat d'Etat ès Sciences Physiques from the university of Toulouse (France) in 1976. In 1980, he became Professor at the Institut National des Sciences Appliquées de Toulouse. He has been in charge of the "Microstructures et Microsystèmes Intégrés (M2I)" group from 1992 to 1997 and has been assistant director for the LAAS-CNRS from 1997 to 2003. He is working on the development of chemical sensors.

FIGURE CAPTIONS

Figure 1: description of the Si_3N_4/PVA/electrolyte structure

Figure 2: creatinine concentration [S](x,t)

Figure 3: ammonia concentration $[NH_3](x,t)$

Figure 4: pH(x,t) function

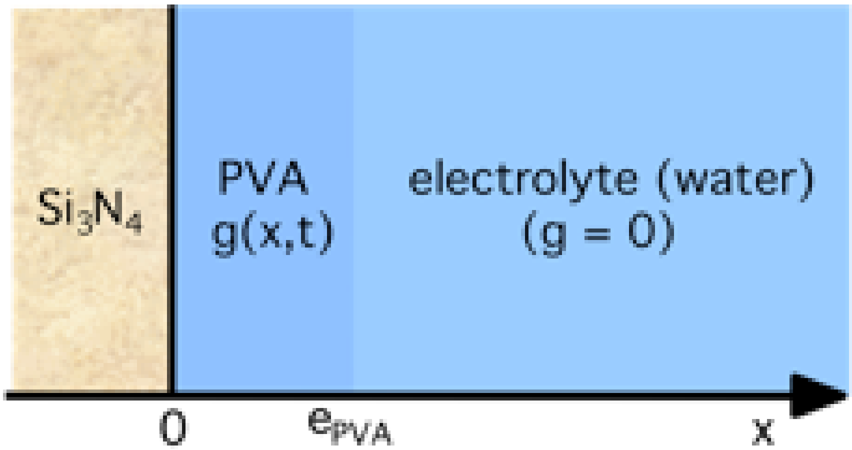
Figure 5: pH temporal variations at the pH-ISFET sensitive surface

Figure 6: creatinine EnFET responses with CD-ase Michaelis constant K_M

Figure 7: creatinine EnFET responses with number of enzymatic units per volume unit n_{enz}

Figure 8: creatinine EnFET responses with solution initial pH value pH_0

Figure 9: Creatinine-EnFET responses: comparison between modelling and experience



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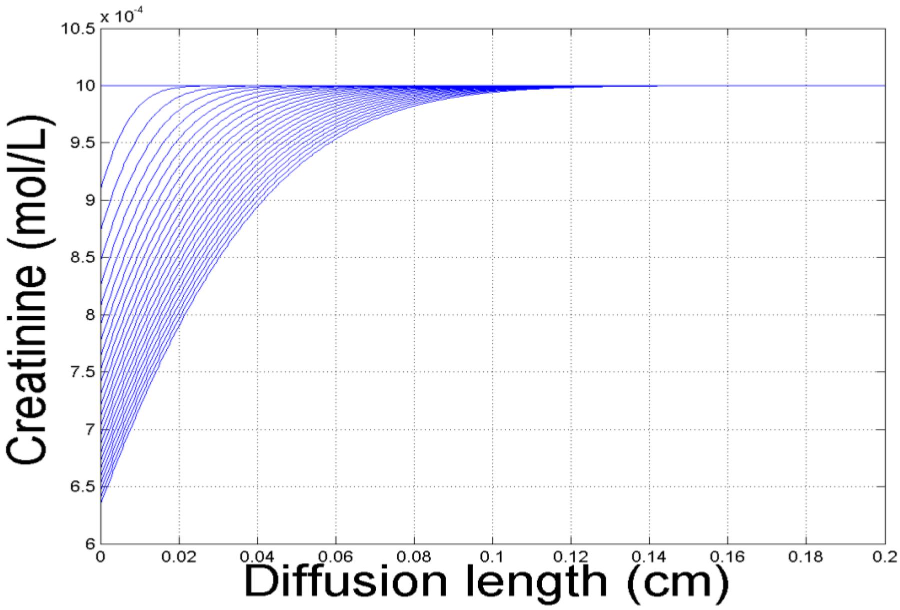


Figure 2: creatinine concentration [S](x,t)

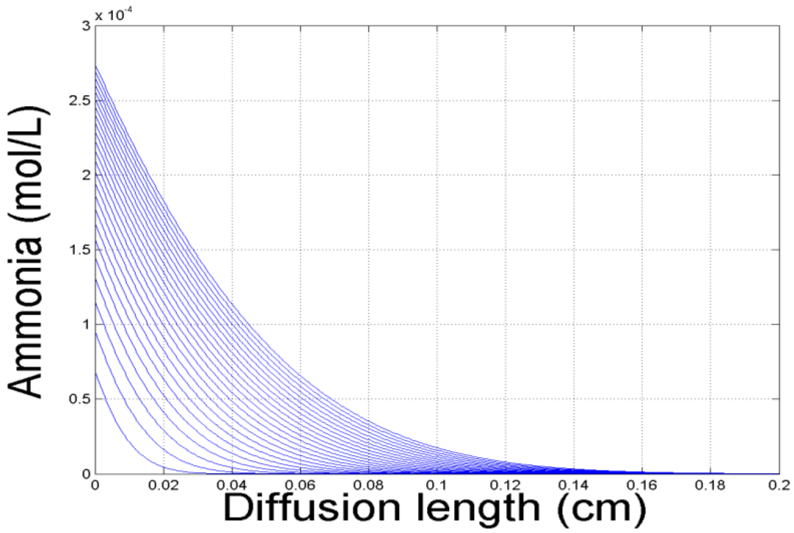


Figure 3: ammonia concentration $[NH_3](x,t)$

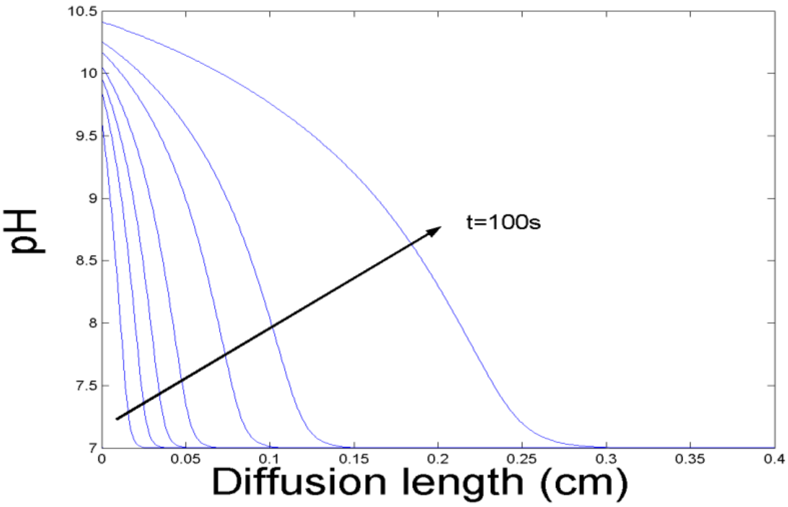


Figure 4: pH(x,t) function

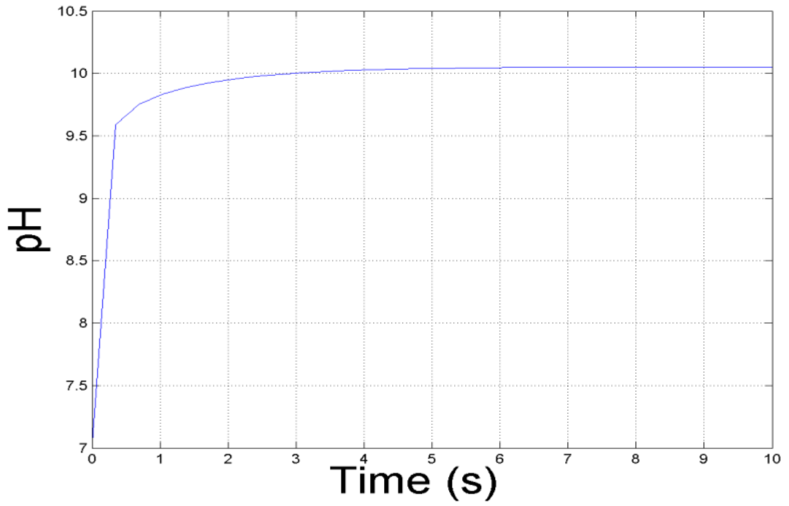
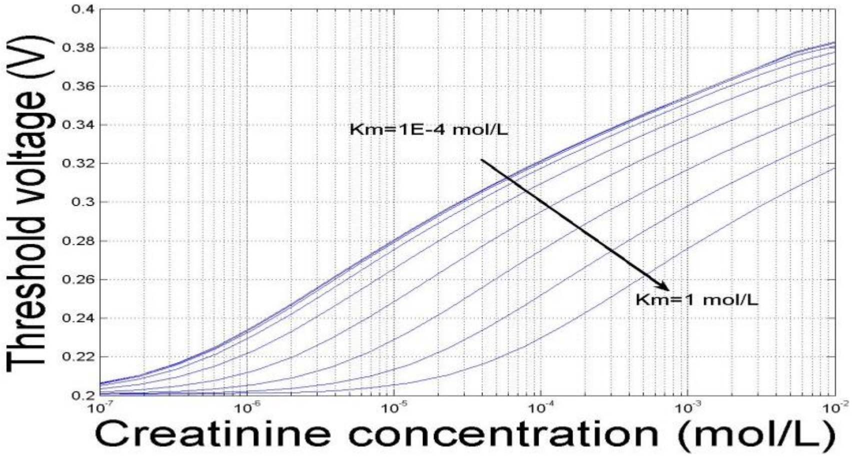


Figure 5: pH temporal variations at the pH-ISFET sensitive surface



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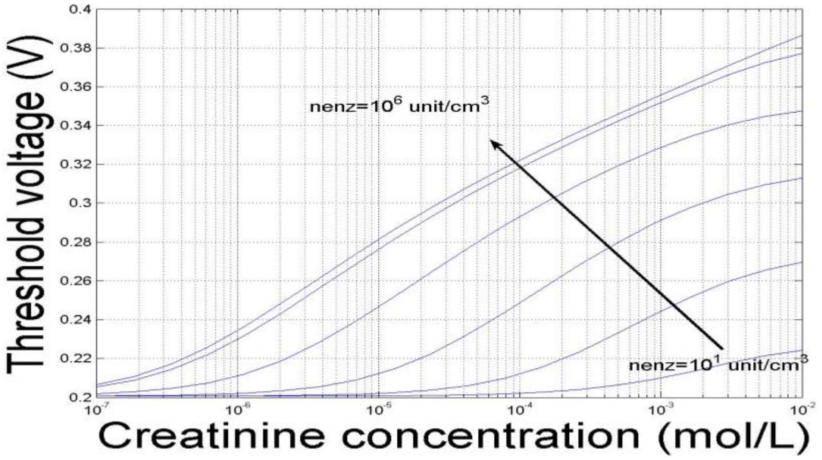

Figure 7: creatinine EnFET responses with number of enzymatic units per volume unit n_{enz}

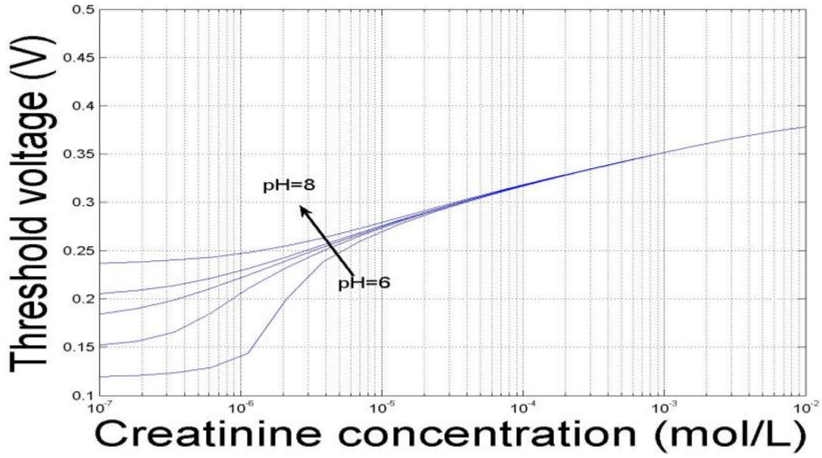

Figure 8: creatinine EnFET responses with solution initial pH value pH_0

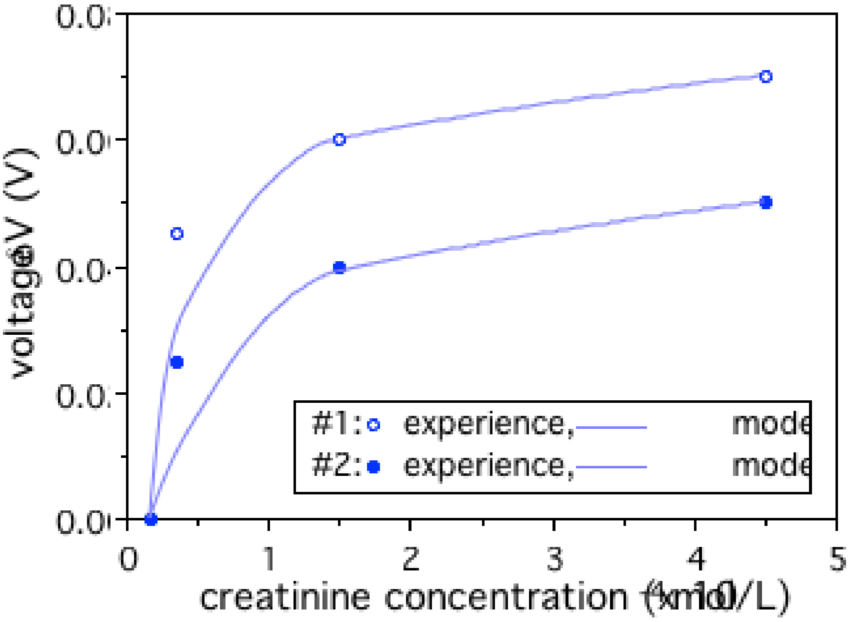

Figure 9: Creatinine-EnFET responses: comparison between modelling and experience